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A·D

PCT/GB99/01509

,TENT COOPERATION TRE Y

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 14 January 2000 (14.01.00)	
International application No. PCT/GB99/01509	**Applicant's or agent's file reference**
International filing date (day/month/year) 01 June 1999 (01.06.99)	**Priority date** (day/month/year) 29 May 1998 (29.05.98)
Applicant TISDALE, Michael, John et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

15 December 1999 (15.12.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Jean-Marc Vivet
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/331 (July 1992)

3052192

PATENT COOPERATION TREATY

n the
...TERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

WILSON GUNN SKERRETT
Charles House
148/9 Great Charles Street
Birmingham B3 3HT
GRANDE BRETAGNE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

	Date of mailing *(day/month/year)* 06.09.2000
Applicant's or agent's file reference JNHS	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/01509	01/06/1999	29/05/1998

Applicant
TISDALE, Michael, John et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Sinanovic, E Tel.+31 70 340-2672

Form PCT/IPEA/416 (July 1992)

RECEIVED - 8 SEP 2000

PATENT COOPERATION TREATY

PCT

REC'D 11 SEP 2000
WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
JNHS		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/01509	01/06/1999	29/05/1998
International Patent Classification (IPC) or national classification and IPC C07K14/00		
Applicant TISDALE, Michael, John et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
15/12/1999	06.09.2000
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer Masturzo, P Telephone No. +31 70 340 2275

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/01509

I. Basis of th rep rt

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-44 as originally filed

Claims, No.:

1-30 as received on 07/07/2000 with letter of 07/07/2000

Drawings, sheets:

1/10-10/10 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-30
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-9, 11, 27-28
	No:	Claims	10,12-26,29-30
Industrial applicability (IA)	Yes:	Claims	1-18,21-30
	No:	Claims	19-20

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following document:

D1: Proc. Natl. Acad. Sci. USA 94, pages 4626-4630 (1997).

1) D1 (see page 4627, left column) reveals the existence of both polyclonal and monoclonal antibodies against the lipid mobilizing factor of the present application, which is identified with a known protein. There is however no hint to the use of these antibodies to fight tumors; therefore claims 27 and 28 considered to be novel, as the other claims are, under Art. 33(2) PCT.

2) For the assessment of the present claims 19-20 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

3) The problem underlying the present application consists in the provision of an alternative lipid-mobilizing agent. The problem could be considered as solved by the protein whose sequence is provided in claim 1. Even though demonstration of the activity of one shorter fragment has been disclosed in Figure 12 and page 31, claim 10 concerns all fragments deriving from enzymatic degradation of the lipolytic agent concerned. It is too broad and moreover contradictory with claim 7, where it is stated that chymotrypsin treatment abolishes biological activity. Therefore claim 10 and 12-26, as well as claims 29-30 as far as referred to claim 10, are objected to under Art. 33(3) PCT for plausibility reasons.

Re Item VIII

Certain observations on the international application

Claim 10 is objected to under Art. 6 and Rule 6 PCT, as it appears to be unsupported by the description and contradictory. In fact claim 7 discloses that chymotrypsin abolishes biological activity, whereas all fragments derived from enzymatic fragmentation are covered. Moreover the applicant provides only one example of an active fragment, whereas the very generic term "enzymatic degradation" might cover also shorter fragments derived from a degradation with a less specific protease. Claims 4-5 refer to derivatives of Zn-alpha2-glycoprotein which are only generically defined; their structure is undefined and the modifications brought forth on the parent molecule are only generically defined. These claims are therefore to be considered "open-ended" ones and as such objectionable under Art. 6 and Rule 6 PCT because of their genericity.

CLAIMS

1. A biologically active lipid mobilising agent for use in therapy which has an apparent molecular mass M_r as determined by gel exclusion chromatography greater than 6.0 kDa, and which is capable of inducing lipolysis in mammalian adipocytes, characterised in that it has the properties and characteristics of a Zn-α_2-glycoprotein.

2. A purified biologically active lipid mobilising agent as claimed in Claim 1 for use in therapy characterised in that it is substantially free of proteolytic activity and consists essentially of a glycosylated polypeptide having an apparent relative molecular mass M_r of about 43kDa as determined by its electrophoretic mobility when subjected to 15% SDS-PAGE electrophoresis and having homology in amino acid sequence with the amino acid sequence (SEQ ID No: 1) of human plasma Zn-α_2-glycoprotein.

3. A lipid mobilising agent as claimed in Claim 2 further characterised in that it is obtainable by a process that includes sequential steps of subjecting biological material to ion exchange chromatography, exclusion chromatography, and then to hydrophobic interaction chromatography, said biological material being urine from a cancer cachexia patient or an extract of a culture of a MAC16 tumour cell line deposited under the provisions of the Budapest Treaty in the European Collection Of Animal Cell Cultures (ECACC) under an Accession No. 89030816.

4. A biologically active lipid mobilising agent as claimed in Claim 1 for therapeutic use which is a glycosylated polypeptide wherein the polypeptide moiety is selected from one of the following groups:

a) a polypeptide having the amino acid sequence of a Zn-α_2-glycoprotein;

b) a polypeptide which in respect to (a) is deficient in one or more

amino acids that do not significantly affect the lipid mobilising or lipolytic activity;

c) a polypeptide in which in respect to (a) one or more amino acids are replaced by a different amino acid or acids that do not significantly affect the lipid mobilising or lipolytic activity;

d) a polypeptide in which in respect to (a) there is incorporated a plurality of additional amino acids which do not interfere with the biological lipolytic activity.

5. A biologically active lipid mobilising agent for use in therapy as claimed in Claim 1 consisting essentially of a glycoprotein that has a polypeptide amino acid sequence homologous with the amino acid sequence (SEQ ID No: 1) of human plasma Zn-α_2-glycoprotein, or with a variant thereof which is modified by minor additions, deletions, or substitutions that do not substantially affect its lipid mobilising activity in biological systems.

6. A lipid mobilising agent for use in therapy as claimed in Claim 4 or 5 further characterised in that it has an apparent relative molecular mass M_r of about 43kDa as determined by its electrophorectic mobility when subjected to 15% SDS-PAGE electrophoresis.

7. A lipid mobilising agent for use in therapy as claimed in any one of Claims 1 to 6 further characterised in that when subjected to digestion with chymotrypsin its lipid mobilising properties are destroyed.

8. A lipid mobilising agent for use in therapy as claimed in any one of Claims 1 to 7 further characterised in that it has the potential *in vitro* to stimulate adenylate cyclase activity in a guanine triphosphate (GTP) dependent process upon incubation with murine adipocyte plasma membranes.

9. A lipid mobilising agent for use in therapy as claimed in any one of Claims 1 to 8 further characterised in that it has substantially the same

immunological properties as human Zn-α_2-glycoprotein.

10. A biologically active lipid mobilising agent for use in therapy which is capable of inducing lipolysis in mammalian adipocytes characterised in that it has an apparent molecular mass M_r as determined by gel exclusion chromatograph greater than 6.0kDa and is obtainable by subjecting the lipid mobilising agent claimed in any one of the preceding claims to fragmentation by enzymatic degradation.

11. A biologically active lipid mobilising agent as claimed in Claim 10 for use in therapy that is a fragment of a glycoprotein or glycosylated polypeptide which is a component of the lipid mobilising agent claimed in any one of Claims 1 to 9 produced by digesting the latter with trypsin

12. A lipid mobilising agent for use in therapy as claimed in any one of the preceding claims further characterised in that it is substantially free of proteolytic activity.

13. A lipid mobilising agent for use in therapy as claimed in any one of the preceding claims further characterised in that the polypeptide chain of the polypeptide component has an N-terminus blocked by a pyroglutamate residue.

14. A lipid mobilising agent for use in therapy as claimed in any one of the preceding claims further characterised in that the lipid mobilising activity is destroyed by periodate treatment.

15. Use of a lipid mobilising agent as claimed in any of the preceding claims for the manufacture of a medicament useful in human medicine for treating conditions of overweight or obesity and/or for stimulating muscle development.

16. A method of isolating and purifying a lipid mobilising agent having the properties and characteristics of a Zn-α_2-glycoprotein, said method comprising subjecting an extract of a cachexia-inducing tumour or of a culture of a

cachexia-inducing tumour cell line, or a sample of urine or other body fluid of a mammal bearing a cachexia-inducing tumour, to a combination of ion exchange, gel filtration size exclusion chromatography, and hydrophobic interaction chromatography, and recovering a single product or molecular species having an apparent relative molecular mass of 43kDa, as determined by 15% SDS-PAGE electrophoresis, which is substantially free of proteolytic activity.

17. A pharmaceutical composition for use in treating mammals, said composition containing as the active constituent an effective therapeutic amount of a lipid mobilising agent as claimed in any one of Claims 1 to 14, together with a pharmaceutically acceptable carrier, diluent or excipient.

18. A pharmaceutical composition as claimed in Claim 17 which is an injectable formulation incorporating a carrier in the form of a pharmaceutically acceptable injection vehicle.

19. A method of treating a mammal to bring about a weight reduction or reduction in obesity, said method comprising administering to the mammal in need of such treatment a therapeutically effective dosage of a lipid mobilising agent as claimed in any one of Claims 1 to 14.

20. A method of treating a mammal to bring about a weight reduction or reduction in obesity, said method comprising administering to the mammal in need of such treatment a therapeutically effective dosage of a glycoprotein identical to or homologous with human Zn-α_2-glycoprotein, or an effective lipolytically active fragment thereof which has an apparent molecular mass Mr as determined by gel exclusion chromatography that is greater than 6.0kDa, substantially free of any proteolytic activity.

21. A diagnostic method for detecting the presence of a tumour in a mammal and/or for monitoring the progress of treatment of such a tumour, said

method comprising taking fr m said mammal a sample of urine, blood serum or other body fluid and testing to detect the presence of and/or to measure the amount therein of Zn-α_2-glycoprotein.

22. A diagnostic method as claimed in Claim 21 wherein thc testing is carried out by use of a biochemical reagent capable of specifically recognising and binding to Zn-α_2-glycoprotein.

23. A diagnostic method as claimed in Claim 22 wherein the biochemical reagent is a monoclonal or polyclonal antibody.

24. A diagnostic method as claimed in any one of Claims 21 to 23 further characterised in that it is applied to a sample of urine.

25. A diagnostic kit for carrying out the method of Claim 21 or 22, said kit comprising a receptacle for receiving the sample of body fluid, a biochemical reagent for detecting Zn-α_2-glycoprotein, and instructions for use of said kit.

26. Use of a lipid mobilising agent as defined in any one of Claims 1 to 14 for producing antibodies for use as a diagnostic detecting agent for use in therapy as inhibitors or antagonists to the lipid mobilising agent causing cachexia in cancer patients.

27. Use of a preparation of antibodies for the manufacture of a medical preparation or medicament for the treatment of cachexia-associated cancer and/or tumours, wherein said antibodies are capable of specifically recognising and binding to the lipid mobilising agent claimed in any one of Claims 1 to 14.

28. Use as claimed in Claim 27 of a preparation of antibodies wherein thc antibodies are monoclonal antibodies.

29. Use of a lipid mobilising agent as defined in any one of Claims 1 to 14 for screening and identifying and/or for carrying out investigations of possible lipolytic activity inhibiting agents having potential as anti-cachectic or antitumour therapeutic agents.

30. Use as claimed in Claim 29 wherein samples of possible antagonists to, or inhibitors of, the activity of said lipid mobilising agent are added to preparations of said lipid mobilising agent, followed by incubation *in vitro* with a preparation of adipocytes and assaying to determine the level of lipolytic activity relative to that of a control sample.

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)*

Box No. I TITLE OF INVENTION

Glycoproteins Having Lipid Mobilising Properties and Therapeutic Applications Thereof

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

TISDALE, MICHAEL JOHN
WELLCOTT
STAR LANE
CLAVERDON
WARWICKSHIRE CV35 8LW
UNITED KINGDOM

[X] This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(that is, country)* of nationality: UNITED KINGDOM

State *(that is, country)* of residence: UNITED KINGDOM

This person is applicant for the purposes of: [X] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

TODOROV, PENIO TODOROV
9 MATTOCK WAY
ABINGDON
OXFORDSHIRE OX14 2PB
UNITED KINGDOM

This person is:

[ ] applicant only

[X] applicant and inventor

[ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is,country)* of nationality: BULGARIA

State *(that is, country)* of residence: UNITED KINGDOM

This person is applicant for the purposes of: [X] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

[ ] Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: [X] agent [ ] common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

H.N. & W.S. SKERRETT
CHARLES HOUSE
148/9 GREAT CHARLES STREET
BIRMINGHAM B3 3HT
UNITED KINGDOM

Telephone No. 0121 236 1038

Facsimile No. 0121 233 2875

Teleprinter No.

[X] **Adress for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- ☒ AP **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ EA **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ EP **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ OA **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- ☒ AL Albania .....
- ☒ AM Armenia .....
- ☒ AT Austria .....
- ☒ AU Australia .....
- ☒ AZ Azerbaijan
- ☒ BA Bosnia and Herzegovina .....
- ☒ BB Barbados
- ☒ BG Bulgaria .....
- ☒ BR Brazil .....
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- ☒ CA Canada
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- ☒ CN China .....
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- ☒ KG Kyrgyzstan .....
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- ☒ KR Republic of Korea .....
- ☒ KZ Kazakhstan .....
- ☒ LC Saint Lucia
- ☒ LK Sri Lanka
- ☒ LR Liberia
- ☒ LS Lesotho .....
- ☒ LT Lithuania
- ☒ LU Luxembourg
- ☒ LV Latvia
- ☒ MD Republic of Moldova .....
- ☒ MG Madagascar .....
- ☒ MK The former Yugoslav Republic of Macedonia .....
- ☒ MN Mongolia
- ☒ MW Malawi .....
- ☒ MX Mexico .....
- ☒ NO Norway
- ☒ NZ New Zealand .....
- ☒ PL Poland .....
- ☒ PT Portugal .....
- ☒ RO Romania
- ☒ RU Russian Federation .....
- ☒ SD Sudan
- ☒ SE Sweden
- ☒ SG Singapore
- ☒ SI Slovenia .....
- ☒ SK Slovakia .....
- ☒ SL Sierra Leone
- ☒ TJ Tajikistan .....
- ☒ TM Turkmenistan .....
- ☒ TR Turkey .....
- ☒ TT Trinidad and Tobago .....
- ☒ UA Ukraine .....
- ☒ UG Uganda .....
- ☒ US United States of America .....
- ☒ UZ Uzbekistan .....
- ☒ VN Viet Nam .....
- ☒ YU Yugoslavia .....
- ☒ ZW Zimbabwe .....

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- ☒ ZA SOUTH AFRICA .....
- ☒ AE UNITED ARAB EMIRATES .....
- ☐ .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box N . VI PRIORITY CLAIM ☐ Further priority claim indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 29 May 1998 (29.05.98)	GB 9811465.5	United Kingdom		
item (2)				
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): 1 (GB 9811465.5)

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):*

ISA /

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets:**

request	: 3
description (excluding sequence listing part)	: 44
claims	: 6
abstract	: 1
drawings	: 10
sequence listing part of description	: 2
Total number of sheets	: 66

This international application is **accompanied by** the item(s) marked below:

1. ☐ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☒ other *(specify)*: Letter

Figure of the drawings which should accompany the abstract:

Language of filing of the international application:

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

MICHAEL JOHN TISDALE and PENIO TODOROV TODOROV

AGENTS

For receiving Office use only

1. Date of actual receipt of the purported international application:	2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority (if two or more are competent): ISA /	6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

CLAIMS

1. A biologically active lipid mobilising agent for use in therapy characterised in that it has the properties and characteristics of a Zn-α_2-glycoprotein, or of a fragment of a Zn-α_2-glycoprotein that has an apparent molecular mass M_r as determined by gel exclusion chromatography greater than 6.0 kDa.

2. A lipid mobilising agent as claimed in Claim 1 for therapeutic use further characterised in that it is a glycosylated polypeptide wherein the polypeptide moiety is selected from one of the following groups:

a) a polypeptide having the amino acid sequence of a Zn-α_2-glycoprotein;

b) a polypeptide which in respect to (a) is deficient in one or more amino acids;

c) a polypeptide in which in respect to (a) one or more amino acids are replaced by a different amino acid or acids;

d) a polypeptide in which in respect to (a) there is a plurality of additional amino acids which do not interfere with the biological lipolytic activity or which may be readily eliminated;

e) a polypeptide which is an allelic derivative of a polypeptide according to (a).

3. A biologically active lipid mobilising agent for use in therapy consisting essentially of a glycoprotein, or a fragment of said glycoprotein that has an apparent relative molecular mass M_r, as determined by gel exclusion chromatography, greater than 6 kDa, said glycoprotein being characterised in that it has a polypeptide amino acid sequence that is homologous with the amino acid sequence (SEQ ID No: 1) of human plasma Zn-α_2-glycoprotein, or with a variant thereof which is modified by additions, deletions, or substitutions

that do not substantially affect its lipid mobilising activity in biological systems.

4. A purified biologically active lipid mobilising agent for use in therapy characterised in that it consists essentially of a glycosylated polypeptide comprising a single main component having an apparent relative molecular mass M_r of about 43kDa as determined by its electrophoretic mobility when subjected to 15% SDS-PAGE electrophoresis and having homology in amino acid sequence with the amino acid sequence (SEQ ID No: 1) of human plasma Zn-α_2-glycoprotein.

5. A lipid mobilising agent as claimed in Claim 4 further characterised in that it is obtainable by a process that includes sequential steps of subjecting biological material to ion exchange chromatography, exclusion chromatography, and then to hydrophobic interaction chromatography, said biological material being a body fluid of a cancer cachexia patient or an extract of a culture of a MAC16 tumour cell line deposited under the provisions of the Budapest Treaty in the European Collection Of Animal Cell Cultures (ECACC) under an Accession No. 89030816.

6. A lipid mobilising agent for use in therapy as claimed in any one of Claims 1 to 3 further characterised in that it has an apparent relative molecular mass M_r of about 43kDa as determined by its electrophorectic mobility when subjected to 15% SDS-PAGE electrophoresis.

7. A lipid mobilising agent for use in therapy as claimed in any one of Claims 4 to 6 further characterised in that when subjected to digestion with chymotrypsin, it is fragmented and its lipid mobilising properties are destroyed.

8. A lipid mobilising agent for use in therapy as claimed in any one of Claims 4 to 7 further characterised in that it has the potential *in vitro* to stimulate adenylate cyclase activity in a guanine triphosphate (GTP) dependent

process upon incubation with murine adipocyte plasma membranes.

9. A lipid mobilising agent for use in therapy as claimed in any one of Claims 4 to 8 further characterised in that it has substantially the same immunological properties as human Zn-α_2-glycoprotein.

10. A biologically active lipid mobilising agent for use in therapy that is a fragment of the glycoprotein or glycosylated polypeptide of the lipid mobilising agent claimed in any one of Claims 4 to 9.

11. A biologically active lipid mobilising agent for use in therapy that is a fragment of the glycoprotein or glycosylated polypeptide of the lipid mobilising agent claimed in any one of Claims 4 to 9, said fragment being a product of digesting said glycoprotein or glycosylated polypeptide with trypsin

12. A lipid mobilising agent for use in therapy as claimed in any one of the preceding claims further characterised in that it is substantially free of proteolytic activity.

13. A lipid mobilising agent for use in therapy as claimed in any one of the preceding claims further characterised in that the polypeptide chain of the polypeptide component has an N-terminus blocked by a pyroglutamate residue.

14. A lipid mobilising agent for use in therapy as claimed in any one of the preceding claims further characterised in that the lipid mobilising activity is destroyed by periodate treatment.

15. Use of a lipid mobilising agent as claimed in any of the preceding claims, or a therapeutically effective fragment derived therefrom, for the manufacture of a medicament useful in human medicine for treating conditions of overweight or obesity and/or for stimulating muscle development.

16. A method of isolating and purifying a lipid mobilising agent having the properties and characteristics of a Zn-α_2-glycoprotein, said method comprising subjecting an extract of a cachexia-inducing tumour or of a culture of a

cachexia-inducing tumour cell line, or a sample of urine or other body fluid of a mammal bearing a cachexia-inducing tumour, to a combination of ion exchange, gel filtration size exclusion chromatography, and hydrophobic interaction chromatography, and recovering a single product or molecular species having an apparent relative molecular mass of 43kDa, as determined by 15% SDS-PAGE electrophoresis, which is substantially free of proteolytic activity.

17. A pharmaceutical composition for use in treating mammals, said composition containing as the active constituent an effective therapeutic amount of a lipid mobilising agent as claimed in any one of Claims 1 to 14, together with a pharmaceutically acceptable carrier, diluent or excipient.

18. A pharmaceutical composition as claimed in Claim 17 which is an injectable formulation incorporating a carrier in the form of a pharmaceutically acceptable injection vehicle.

19. A method of treating a mammal to bring about a weight reduction or reduction in obesity, said method comprising administering to the mammal a therapeutically effective dosage of a lipid mobilising agent as claimed in any one of Claims 1 to 14.

20. A method of treating a mammal to bring about a weight reduction or reduction in obesity, said method comprising administering to the mammal a therapeutically effective dosage of a glycoprotein identical to or homologous with human Zn-α_2-glycoprotein, or an effective fragment thereof, substantially free of any proteolytic activity.

21. A diagnostic method for detecting the presence of a tumour in mammals and/or for monitoring the progress of treatment of such a tumour, said method comprising taking from said mammal a sample of urine, blood serum or other body fluid and testing to detect the presence of and/or to

measure the amount therein of the lipid mobilising agent claimed in any one of Claims 1 to 14 or of Zn-α_2-glycoprotein.

22. A diagnostic method as claimed in Claim 21 wherein the testing is carried out by use of a biochemical reagent capable of specifically recognising and binding to Zn-α_2-glycoprotein.

23. A diagnostic method as claimed in Claim 22 wherein the biochemical reagent is a monoclonal or polyclonal antibody.

24. A diagnostic method as claimed in any one of Claims 21 to 23 further characterised in that it is applied to a sample of urine.

25. A diagnostic kit for carrying out the method of Claim 21, said kit comprising a receptacle for receiving the sample of body fluid, a biochemical reagent for detecting said lipid mobilising agent or Zn-α_2-glycoprotein, and instructions for use of said kit.

26. Use of a lipid mobilising agent as defined in any one of Claims 1 to 14 for producing antibodies for use as a diagnostic detecting agent for use in therapy as inhibitors or antagonists to the lipid mobilising agent causing cachexia in cancer patients.

27. A preparation of one or more antibodies capable of specifically recognising and binding to the lipid mobilising agent claimed in any one of Claims 1 to 14.

28. A monoclonal antibody to an antigen consisting of the lipid mobilising agent claimed in any one of Claims 1 to 14.

29. Use of the antibody or antibody preparation claimed in Claim 27 or 28 for the manufacture of a medical preparation or medicament for the treatment of cachexia-associated cancer and/or tumours.

30. Use of a lipid mobilising agent as defined in any one of Claims 1 to 14

for screening and identifying and/or for carrying out investigations of possible lipolytic activity inhibiting agents having potential as anti-cachectic or antitumour therapeutic agents.

31. Use as claimed in Claim 30 wherein samples of possible antagonists to, or inhibitors of, the activity of said lipid mobilising agent are added to preparations of said lipid mobilising agent, followed by incubation *in vitro* with a preparation of adipocytes and assaying to determine the level of lipolytic activity relative to that of a control sample.